Coupled analysis of active biological processes for meniscus tissue regeneration

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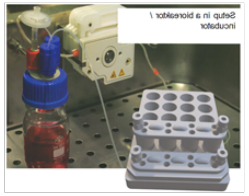
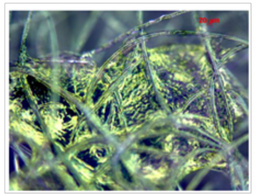
Experimental Study

Scaffold production and cell-seeding





Michael Doser, Alexander Ott, Carsten Linti

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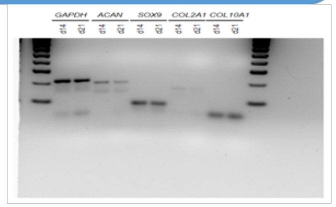
Analysis



Andreas Seitz, Graciosa Teixeira

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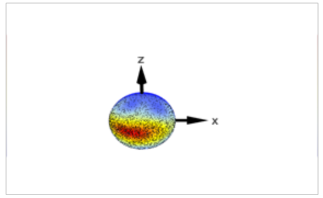
Theoretical Study

Image analysis



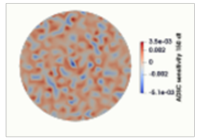
Claudia Redenbach, Alex Keilmann

Modeling and analysis

- PDE-ODE system for cells, hyaluron and ECM,
- fluid(Stokes),
- deformation of tissue

Christina Surulescu, Nishith Mohan

Numerical simulations



Bernd Simeon, Elise Grosjean

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Motivation

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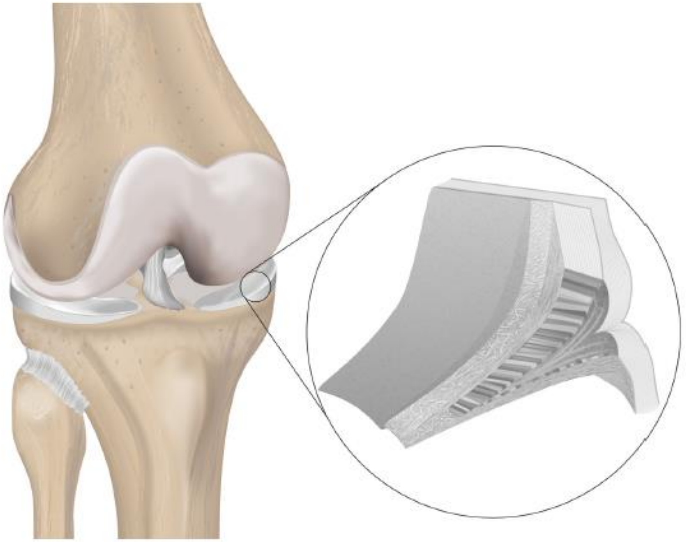
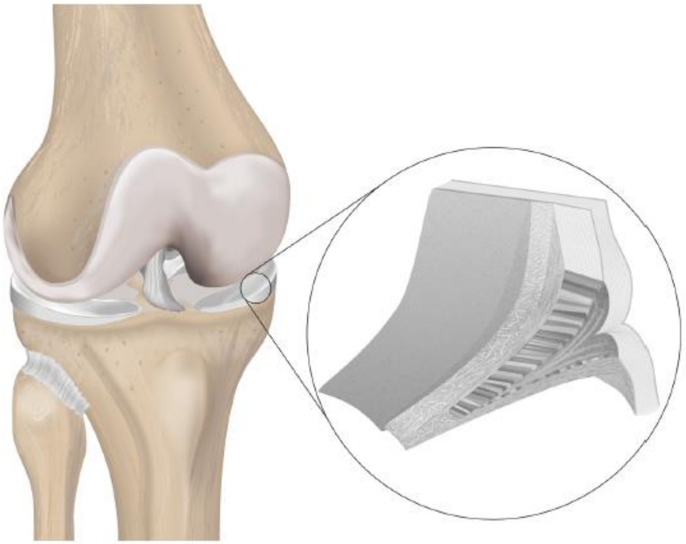


- Meniscectomy leads to premature osteoarthritis of the knee joint
- New paradigm of healing by repair and regeneration of meniscus tissue
- Need for promising substitute
- Replacement tissue for cartilage is successfully generated based on cell cultured scaffolds

Objectives



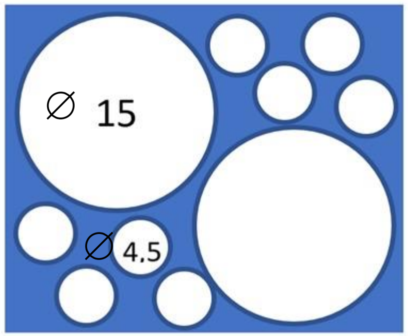
- Experimental study of cell-seeded nonwoven scaffolds in an array of perfusion chambers
- Identification of crucial stimuli for chondrocytes and stem cells (ADSCs) ➔ cell proliferation, differentiation, and migration
- Deduction and study of multiscale models
- Development of efficient numerical methods for coupling of models on several scales and for parameter identification
- Set up of a feedback loop of *in silico* and *in vitro* results to improve modeling and experimental design

Scaffold characterization

Polyethylene terephthalate (PET) needle felts (nonwoven) characterized by:

- Scanning electron microscopy (SEM)
- Micro computed tomography (µCT)
- Indentation mapping
- Multi-step confined compression relaxation test
- Unconfined compression creep test

267-292 g, 25x30 mm^2



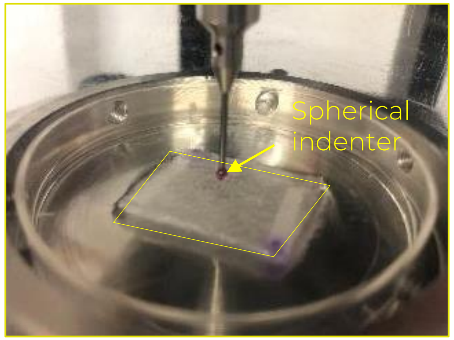
preferencial direction of fibers

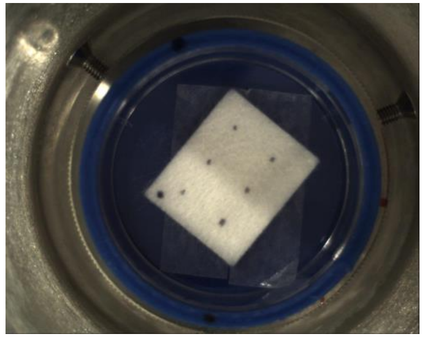
Characterization of the biomechanical performance of the scaffolds

I: Indentation mapping (dry vs. hydrated in 10 mL PBS for 2h)

- N = 6 samples, 6 measuring points/sample
- Indentation amplitude: 15 % h0
- Relaxation time: 10 s
- Spherical indenter: Ø = 5 mm

→ Maximum force (Fmax)

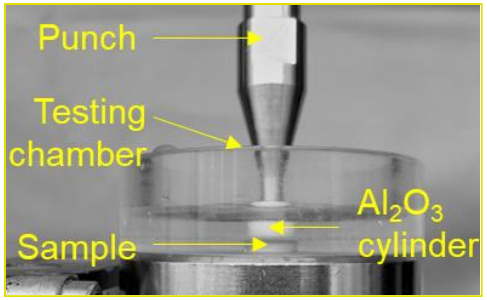


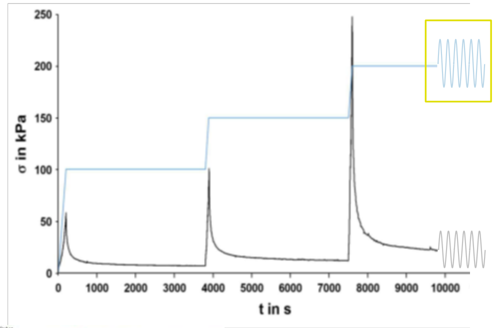


II: Multi-step confined compression relaxation test (Mow et al., 1980)

- N = 6 cylindrical samples Ø 5mm
- 3 consecutive strain levels (ε = 0.1, 0.15, 0.2)
- Relaxation time: 30 minutes

→ Equilibrium Modulus (E_{eq})
→ Permeability of the fiber network (k)

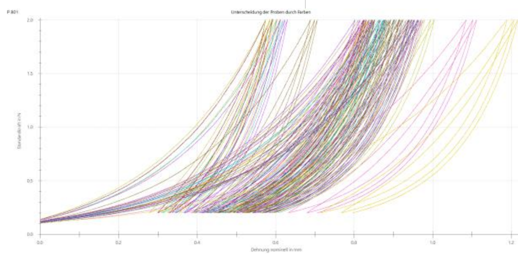




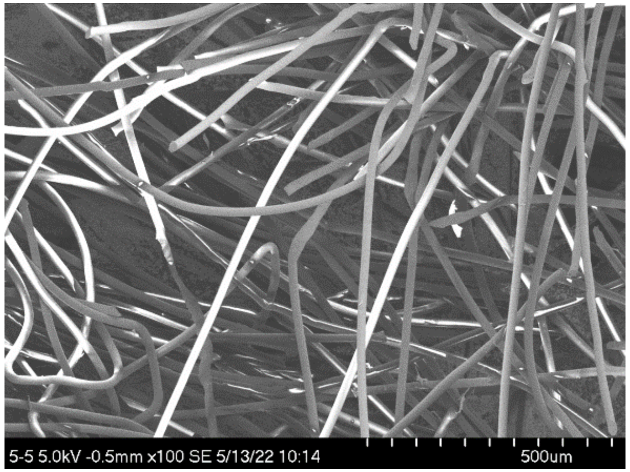
II: Unconfined compression creep test

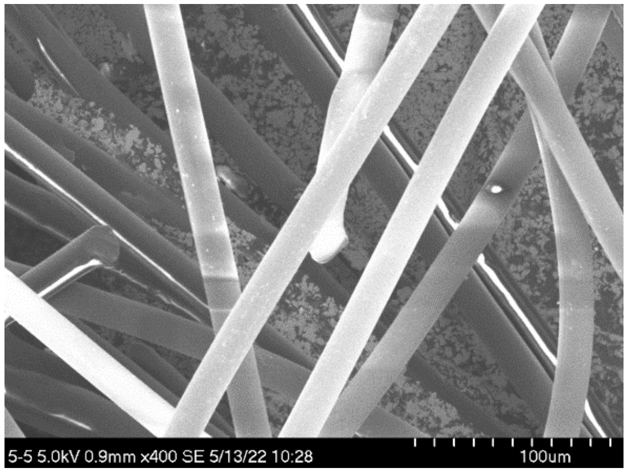
- 2 N maximum force

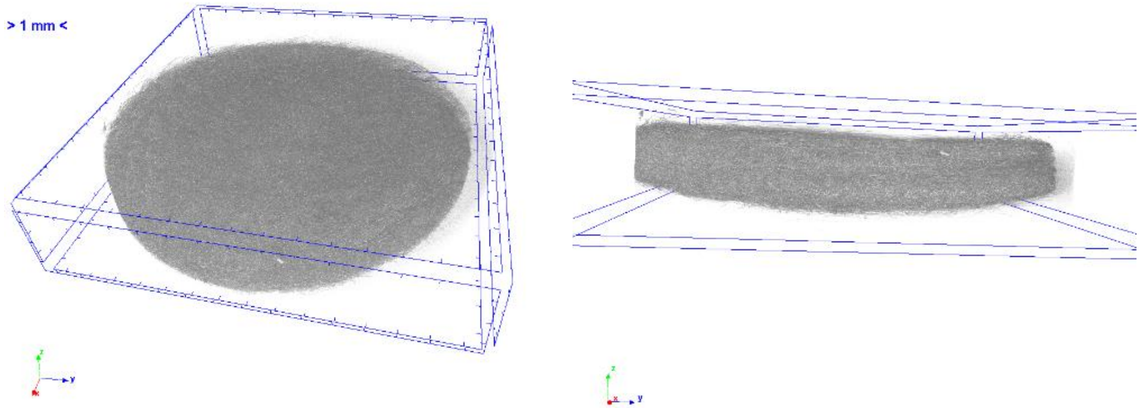
→ Stress-strain diagram to calculate the creep rate



SEM and µCT of PET nonwoven fabrics





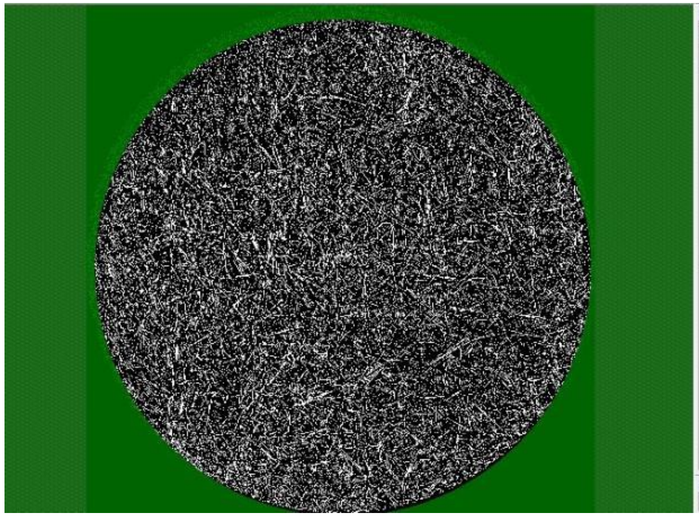


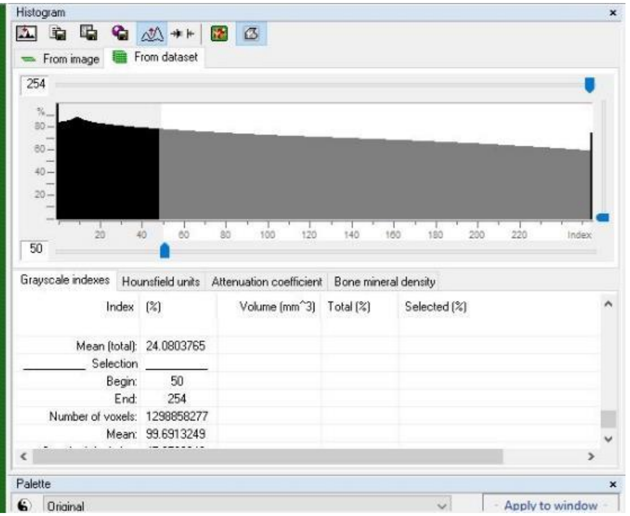
PET grammage: 317 g/m²

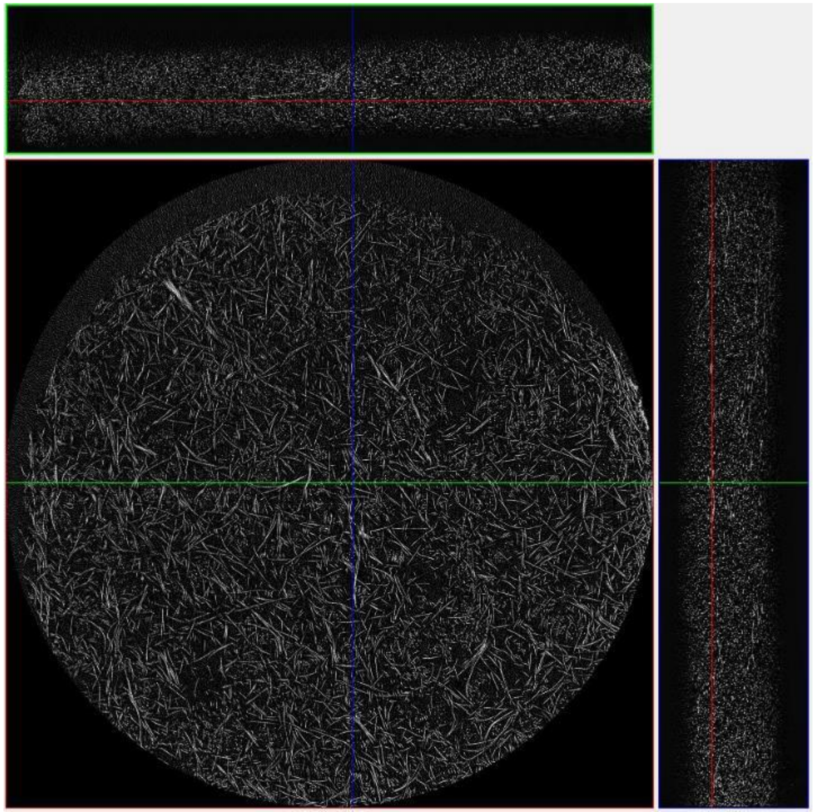
- Textile volume/total volume = 14.85 ± 0.52 %
- **Porosity = 85.15 ± 0.52 %**
- Structure model index (SMI) = 2.35 ± 0.04 %

(SMI = 0 for plates, 3 for rods and 4 for solid spheres)

Thickness distribution of PET fibers







Range (mm)	Mid-range (mm)	Volume (mm³)	Percentage of volume in range (%)
0.00398 - <0.01194	0.00796	19.1	23.3
0.01194 - <0.01989	0.01591	44.8	54.8
0.01989 - <0.02785	0.02387	17.2	21.0
0.02785 - <0.03581	0.03183	0.8	0.9

Indentation mapping

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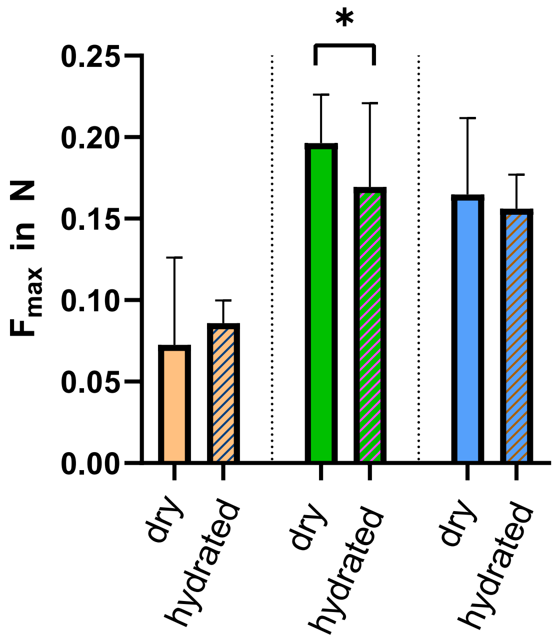
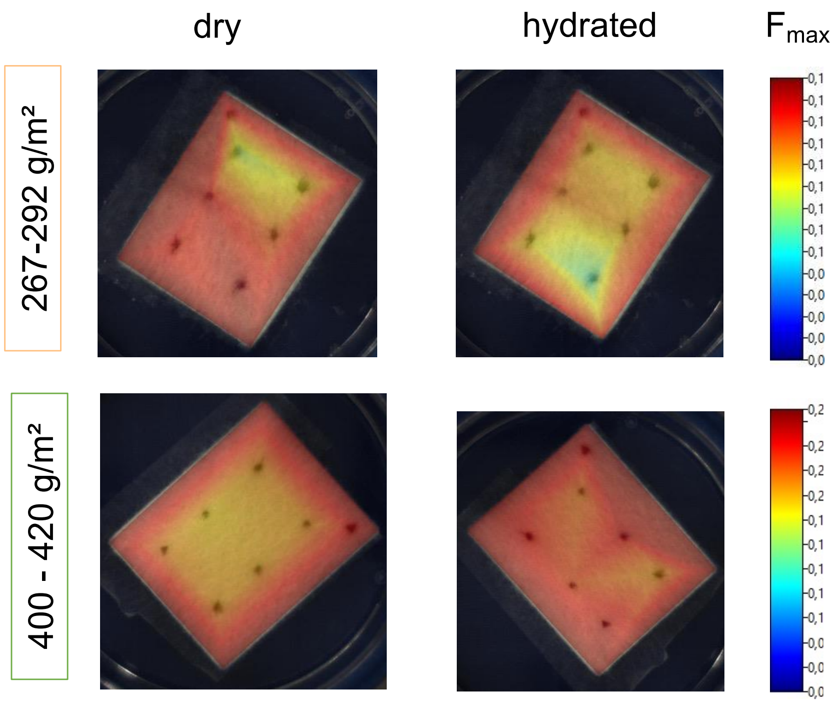


Wilcoxon testing, *p<0.05



267 - 292 g/m²   400 - 420 g/m²   400 - 420 g/m² sterile (25 kGy)

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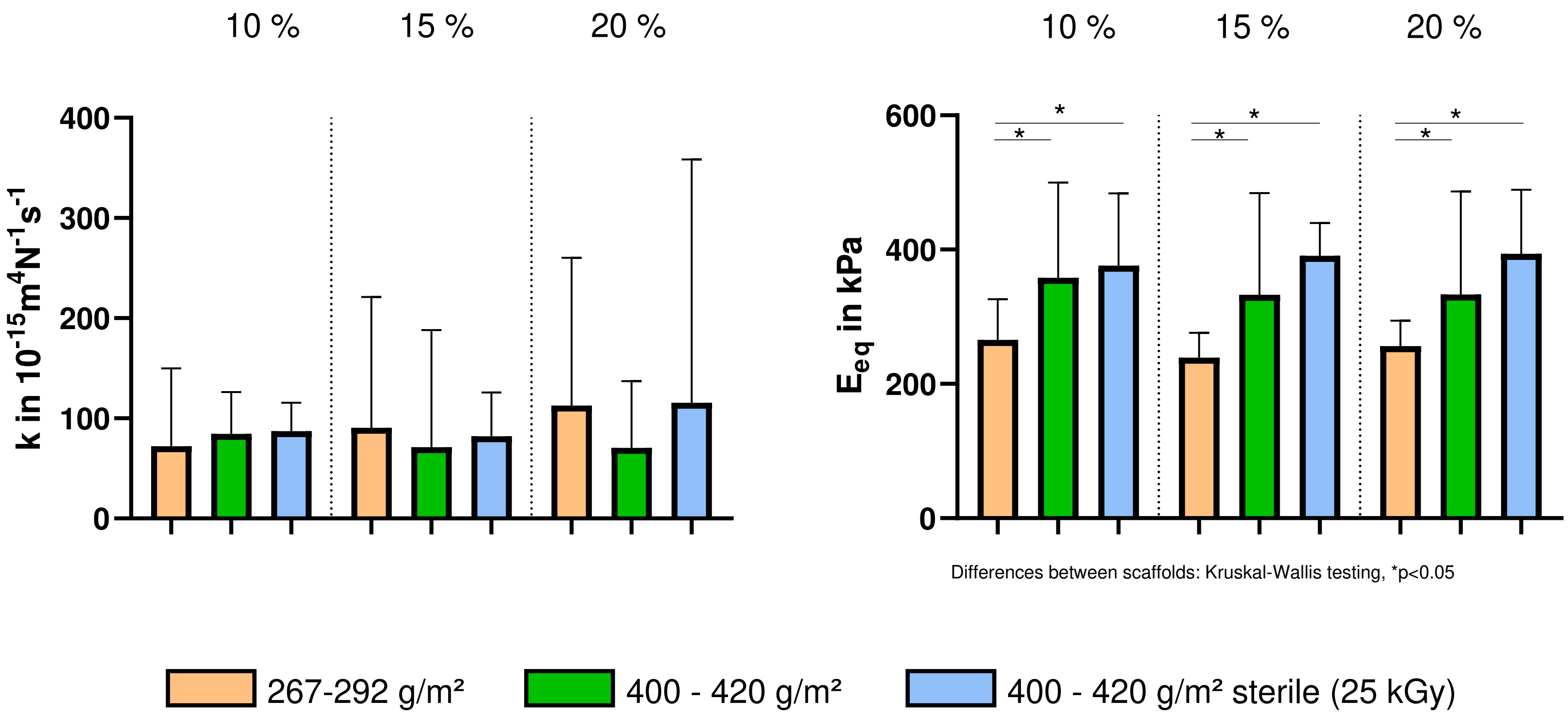
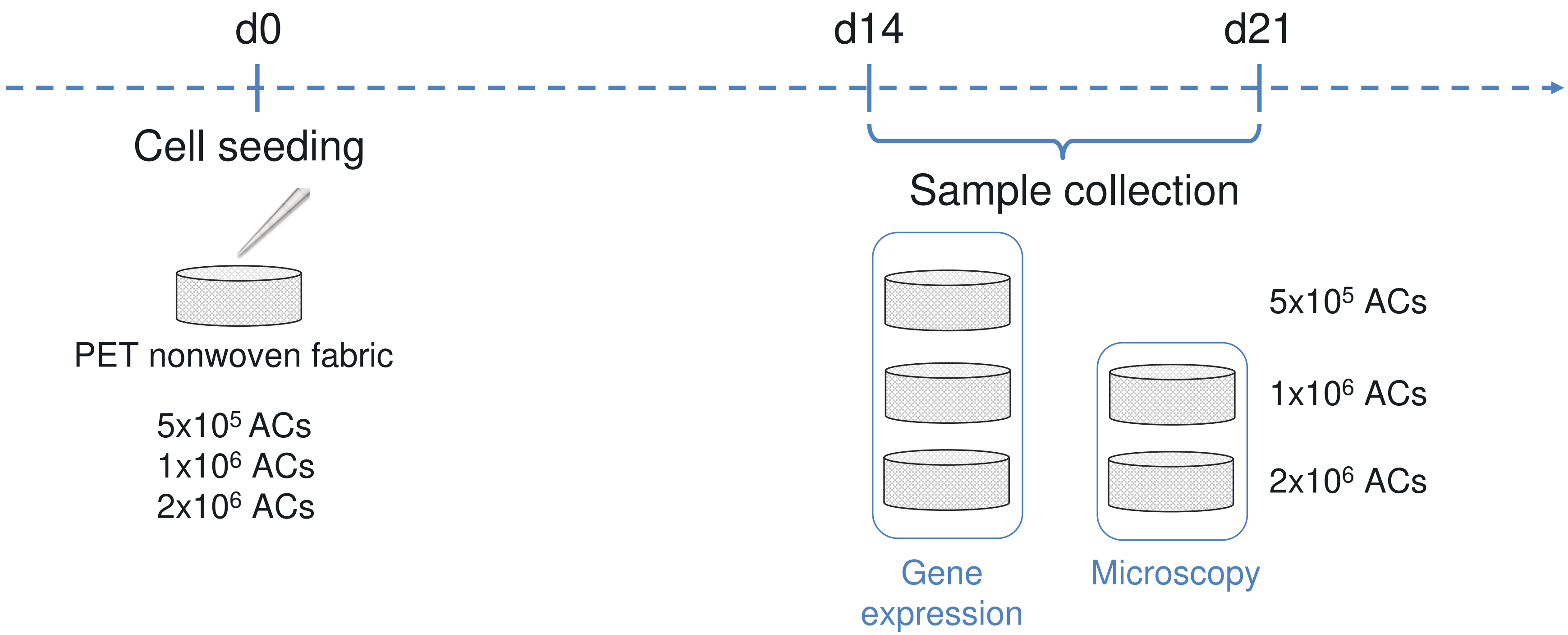
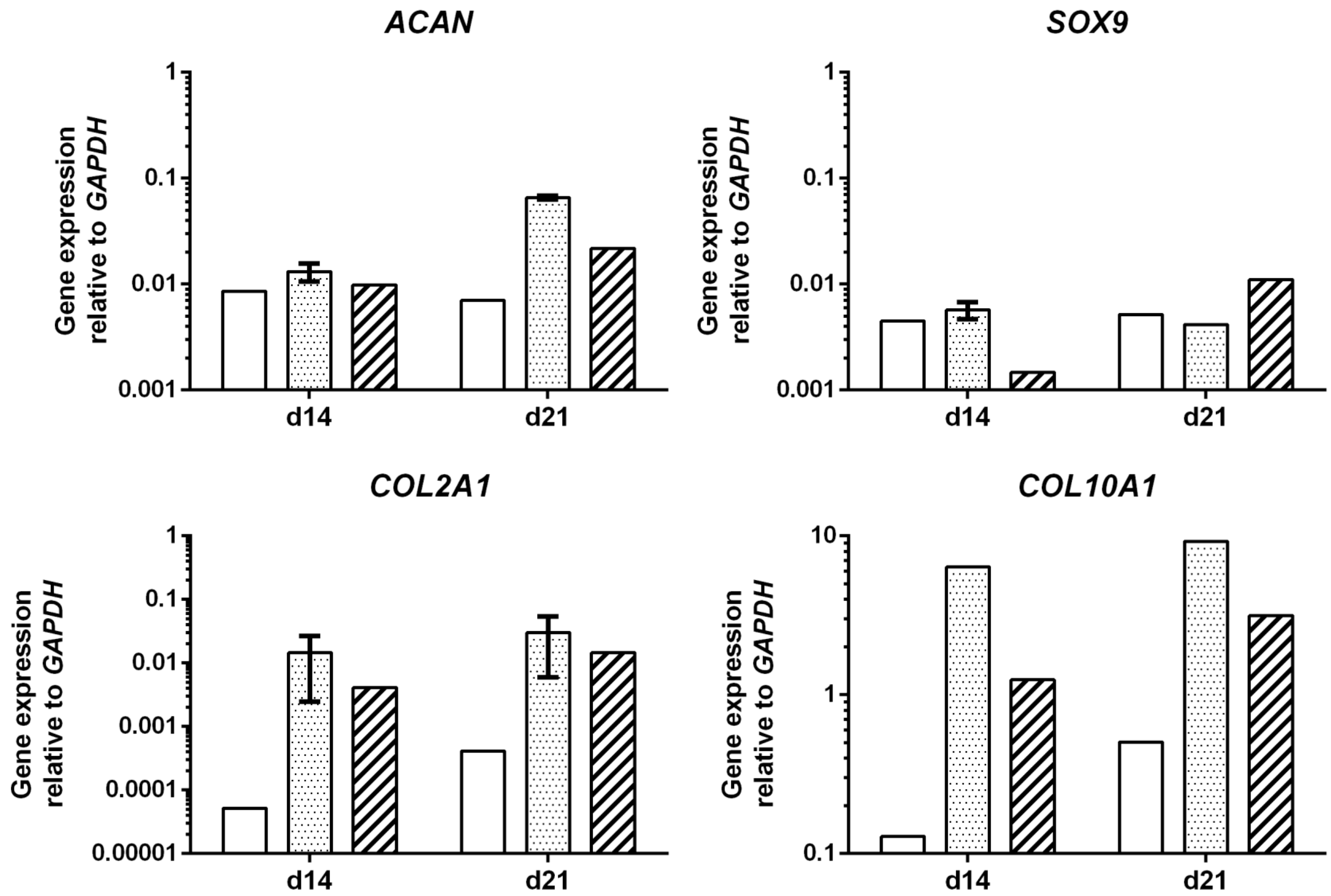
Confined compression



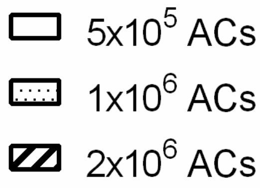
Seeding of articular chondrocytes



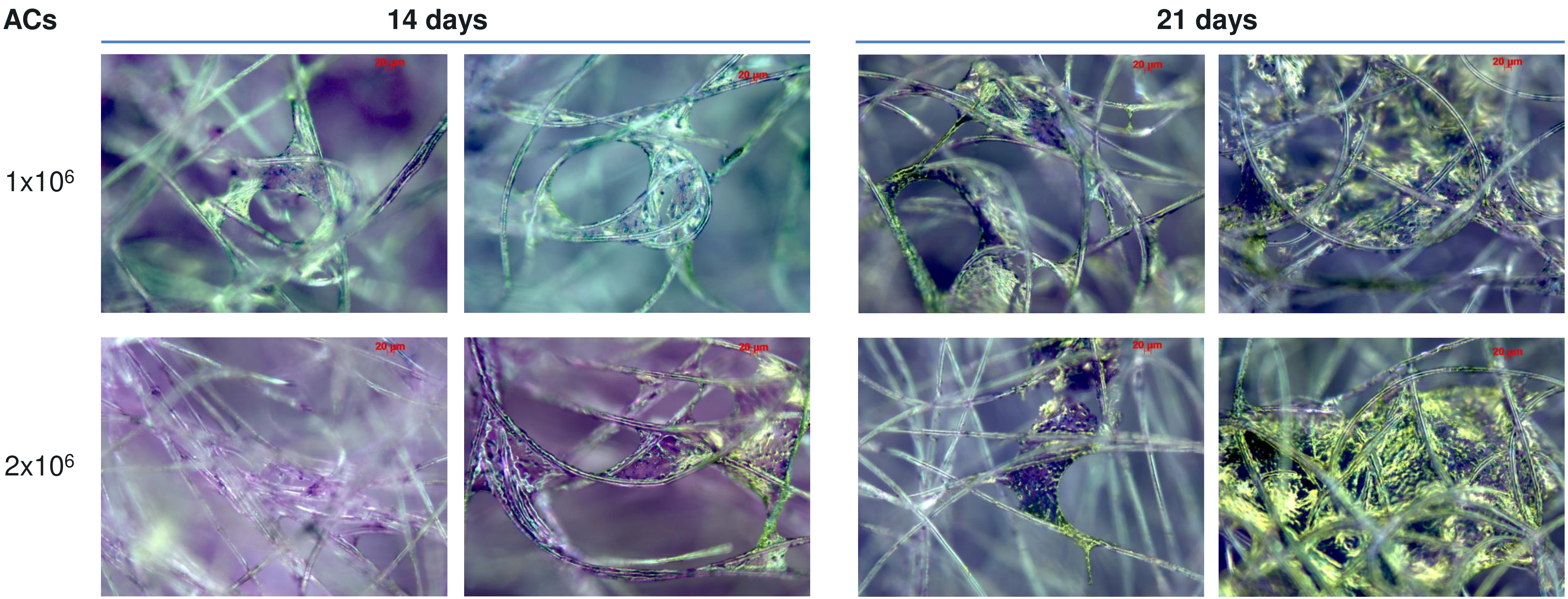
AC gene expression



- **Upregulation of chondrogenic markers** from d14 to d21 (except *SOX9* in 1x10^6 ACs)
- Overall, low expression of the investigated markers

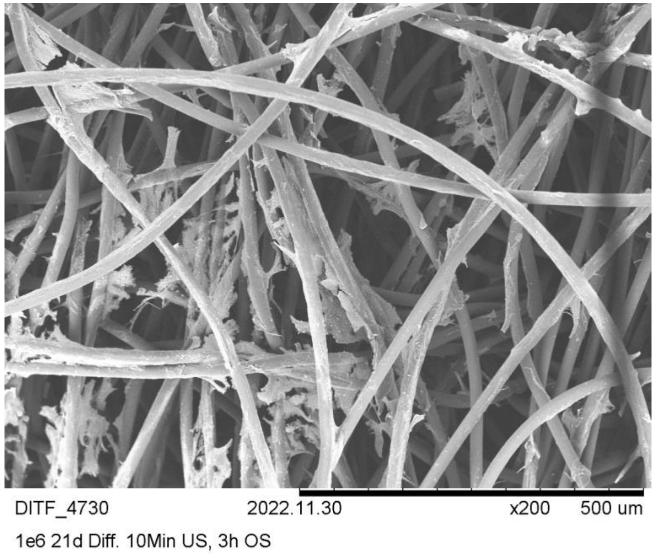


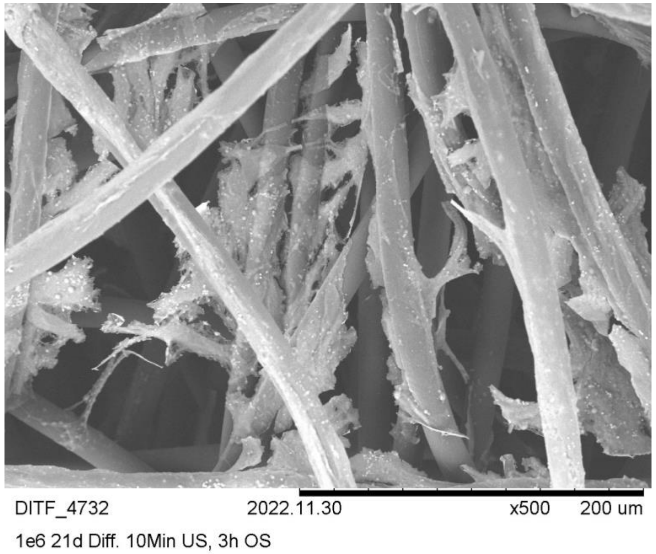
Cell adhesion and proliferation

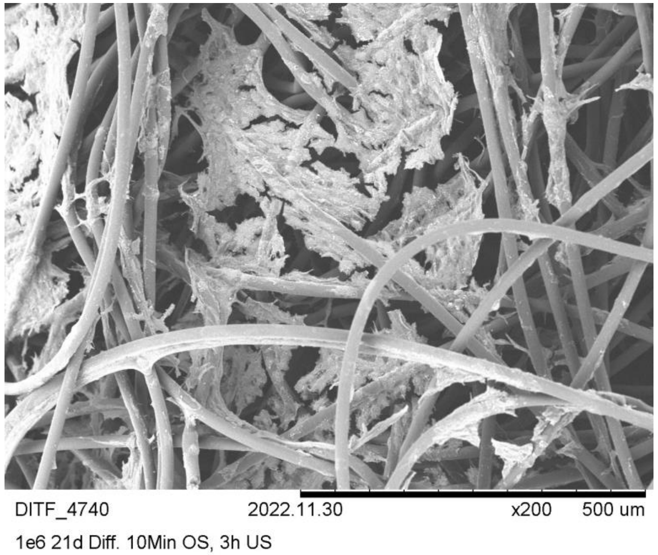


Cell adhesion and proliferation

Scanning electron microscopy (SEM)
$1x10^6$ ACs
day 21







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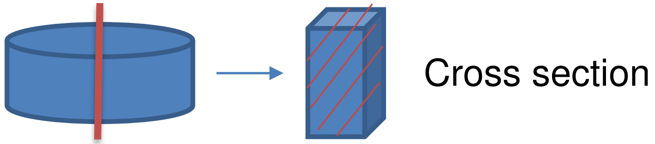
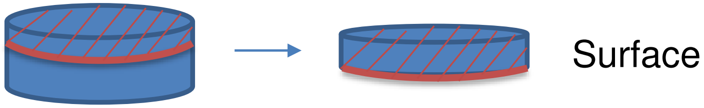
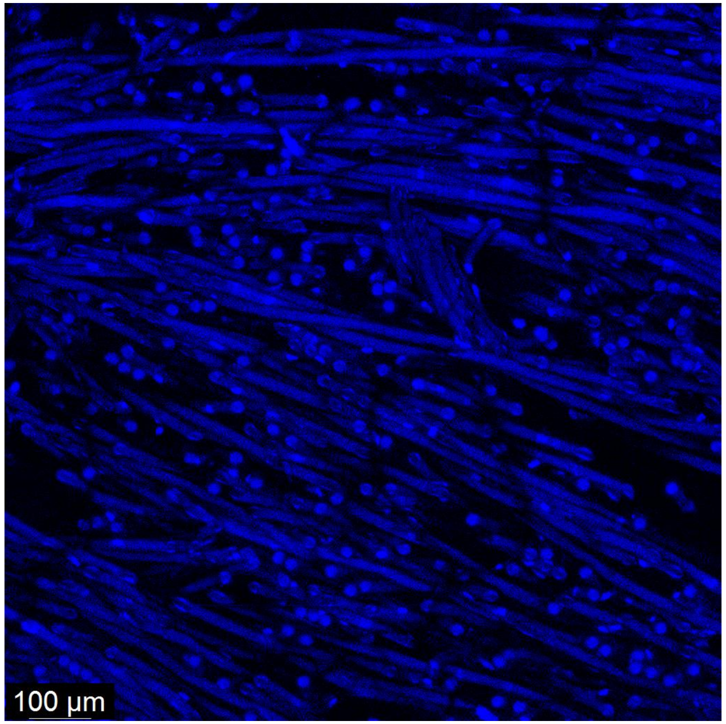
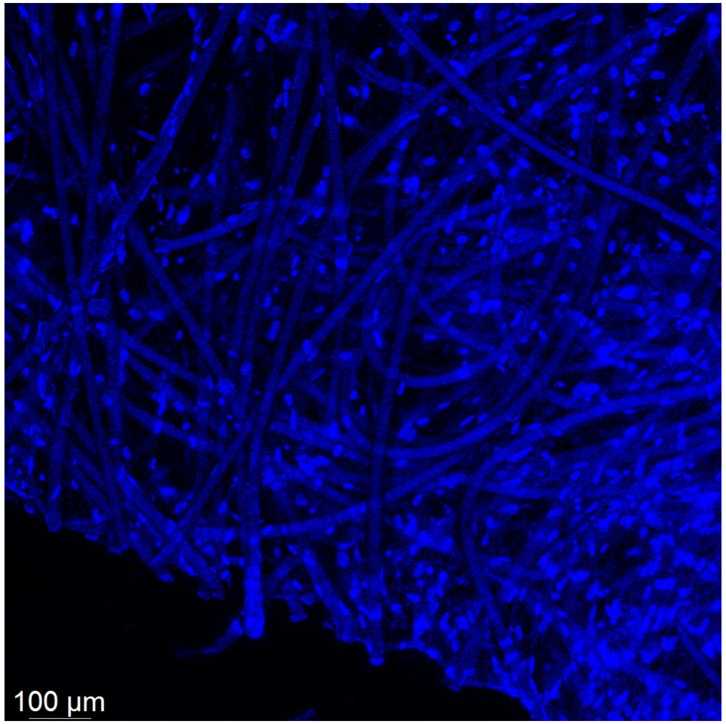








Confocal microscopy, $1x10^6$ ACs, day 21, sections: 200 – 300 µm

Experimental study

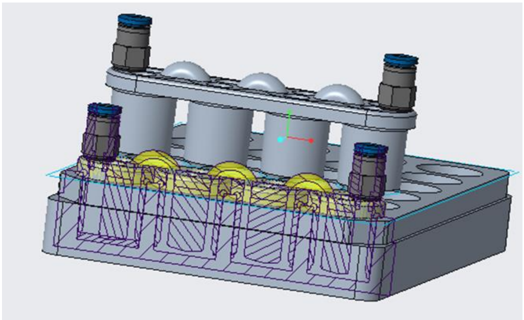
- Successful seeding of scaffolds with ACs
- Adequate culture conditions for cell adhesion and proliferation, but absence of matrix production

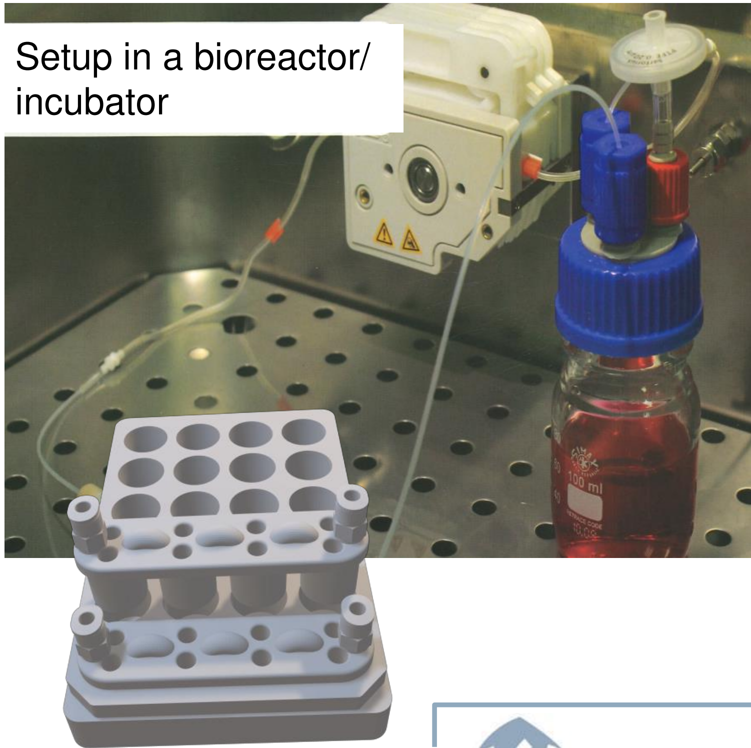
Open research questions:

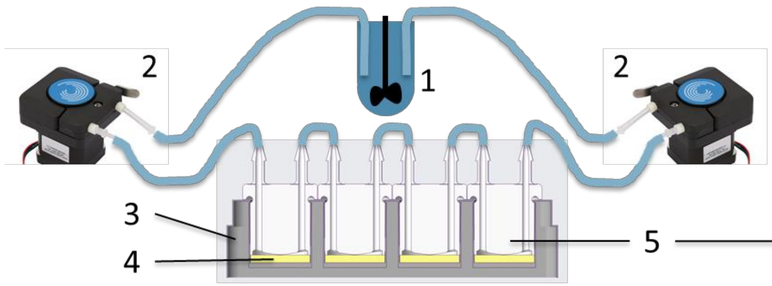
- Differentiation of stem cells before seeding or after seeding inside the scaffolds
- Coating of scaffolds with hyaluranic acid
- Relevant end points
- Differentiation/matrix production under perfusion stress

Perfusion stress chamber

- Development and production of a perfusion chamber for long-term experiments (up to 4 weeks)







1) Bioreactor (medium conditioning & supply)
2) Peristaltic pump
3) 24 well plate
4) Nonwoven scaffold
5) Pressure-Caps

Work flow

Experimental Study

Scaffold production and cell-seeding → **Analysis** →

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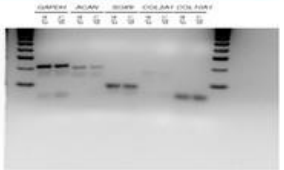

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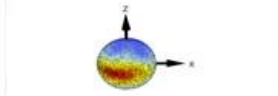
Theoretical Study

Image analysis



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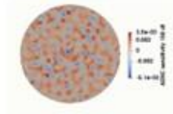
Numerical simulations



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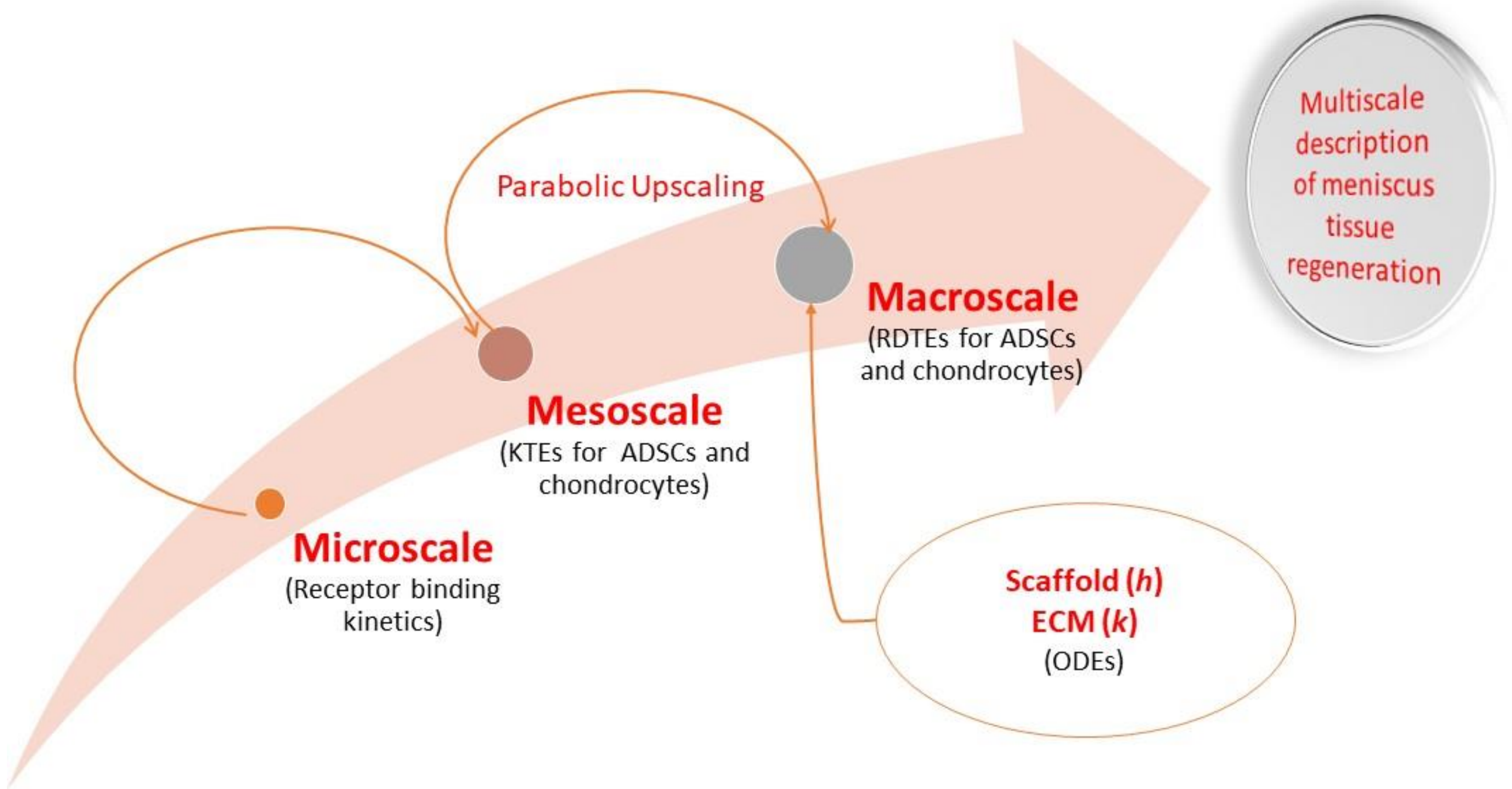
A multiscale approach



Modeling cell migration and (de) differentiation in a scaffold

$$\partial_t c_1 - \nabla\nabla : (\mathbb{D}_1 c_1) + \nabla \cdot \left(\frac{k^- \lambda_{11}}{B(h,k)^2 (B(h,k) + \lambda_{10})} \mathbb{D}_1 \nabla B(h,k) c_1 \right)$$
$$= -\alpha_1(k,S) c_1 + \alpha_2(k,S) \frac{\omega_1}{\omega_2} c_2 + \beta c_1 (1 - c_1 - c_2),$$
$$\partial_t c_2 - \nabla\nabla : (\mathbb{D}_2 c_2) = \alpha_1(k,S) \frac{\omega_2}{\omega_1} c_1 - \alpha_2(k,S) c_2.$$

Macroscopic equations for ADSCs and chondrocytes

Parabolic Upscaling

$$\partial_t p_1 + \nabla_x \cdot (v p_1) + \partial_z (G(z,h,k) p_1) = \mathcal{L}_1[\lambda_1(z)] p_1$$
$$+ \text{(de)differentiation \& proliferation},$$
$$\partial_t p_2 + \nabla_x \cdot (v p_2) = \mathcal{L}_2[\lambda_2] p_2 + \text{(de)differentiation}.$$

KTE for ADSCs and chondrocytes

$$\dot{z} = -z B(h,k) + \frac{\tau^-}{(B(h,k))^2} v \cdot \nabla_x B(h,k) := G(z,h,k),$$
$$\text{with } B(h,k) := \tau_1^+ \frac{h}{H} + \tau_2^+ \frac{k}{K} + \tau^-.$$

Receptor binding kinetics

- c_1 macrpscopic cell density of ADSCs,
- c_2 macroscopic cell density of chondrocytes,
- h: density of hyaluron,
- k: density of ECM.

Modeling cell migration and (de) differentiation in a scaffold

$$\partial_t c_1 - \nabla\nabla : (\mathbb{D}_1 c_1) + \nabla \cdot \left(\frac{k^- \lambda_{11}}{B(h,k)^2 (B(h,k) + \lambda_{10})} \mathbb{D}_1 \nabla B(h,k) c_1 \right) =$$
$$- \alpha_1(k,S) c_1 + \alpha_2(k,S) \frac{\omega_1}{\omega_2} c_2 + \beta c_1 (1 - c_1 - c_2),$$
$$\partial_t c_2 - \nabla\nabla : (\mathbb{D}_2 c_2) = \alpha_1(k,S) \frac{\omega_2}{\omega_1} c_1 - \alpha_2(k,S) c_2.$$

Macroscopic equations for ADSCs and chondrocytes

$\mathbb{D}_i, i = \{1,2\}$- encodes the orientation distribution of scaffold fibers

$$\nabla\nabla : (\mathbb{D}_i c_i) = \nabla \cdot (\mathbb{D}_i \nabla c_i + c_i \nabla \cdot \mathbb{D}), \quad i = \{1,2\}$$

$$\mathbb{D}_1(x) = \frac{1}{\lambda_{10}} \int_{V_1} v \otimes v \frac{q(x,\hat{v})}{\omega_1} dv, \quad \text{and}$$
$$\mathbb{D}_2(x) = \frac{1}{\lambda_2} \int_{V_2} v \otimes v \frac{q(x,\hat{v})}{\omega_2} dv = \frac{\lambda_{10}}{\lambda_2} \left(\frac{\omega_2}{\omega_1} \right)^{\frac{2}{n-1}} \mathbb{D}_1(x).$$

Complete model

$$\partial_t c_1 - \nabla\nabla : (\mathbb{D}_1 c_1) + \nabla \cdot \left(\frac{k^- \lambda_{11}}{B(h,k)^2 (B(h,k) + \lambda_{10})} \mathbb{D}_1 \nabla B(h,k) c_1 \right)$$
$$= -\alpha_1(k,S) c_1 + \alpha_2(k,S) \frac{\omega_1}{\omega_2} c_2 + \beta c_1 (1 - c_1 - c_2),$$

$$\partial_t c_2 - \nabla\nabla : (\mathbb{D}_2 c_2) = \alpha_1(k,S) \frac{\omega_2}{\omega_1} c_1 - \alpha_2(k,S) c_2,$$

$$\partial_t h = -\gamma_1 h c_2 + \frac{c_2}{1 + c_2},$$

$$\partial_t k = -\delta_1 c_1 k + c_2.$$

Macroscopic equations for cell dynamics

$$\rho_s \partial_{tt} \eta_p - \nabla \cdot \sigma_p(\eta_p, p_p) = 0$$

$$\partial_t \left(\frac{1}{M} p_p + \nabla \cdot (\alpha \eta_p) \right) + \nabla \cdot \mathbf{u}_p = 0.$$

Biot's equations

$$\mathbf{u}_p = -\mathbf{K}(\nabla p - \rho_f \mathbf{g})/\mu.$$

Darcy law

$$\rho_f \partial_t \mathbf{u}_f - \nabla \cdot \sigma_f(\mathbf{u}_f, p_f) = 0, \quad \text{and } \nabla \cdot \mathbf{u}_f = 0, \quad \text{in } \Omega_f.$$

Unsteady Stokes equation

$$\sigma_p(n_p, p_p) = \sigma_e(\eta_p) - \alpha p_p I, \quad \text{and } \sigma_e(\eta_p) = \lambda_p (\nabla \cdot \eta_p) I + 2\mu_p D(\eta_p)$$

Equation for stress

A simplified macroscopic model for meniscus tissue regeneration

$$\partial_t c_1 = a_1 \Delta c_1 - \nabla \cdot (b_1 c_1 \nabla h) - \nabla \cdot (b_2 c_1 \nabla k) - \alpha_1(k) c_1 + \alpha_2(k) c_2 + \beta c_1 (1 - c_1 - c_2 - k),$$

$$\partial_t c_2 = \Delta c_2 + \alpha_1(k) c_1 - \alpha_2(k) c_2,$$

$$\partial_t h = -\gamma_1 h c_2 + \frac{c_2}{1 + c_2},$$

$$\partial_t k = -\delta_1 c_1 k + c_2,$$

subject to boundary conditions

$$-\frac{\partial c_1}{\partial \nu} + b_1 c_1 \frac{\partial h}{\partial \nu} + b_2 c_1 \frac{\partial k}{\partial \nu} = \frac{\partial c_2}{\partial \nu} = 0 \quad \text{on} \quad \partial\Omega \times (0, T),$$

and, initial conditions

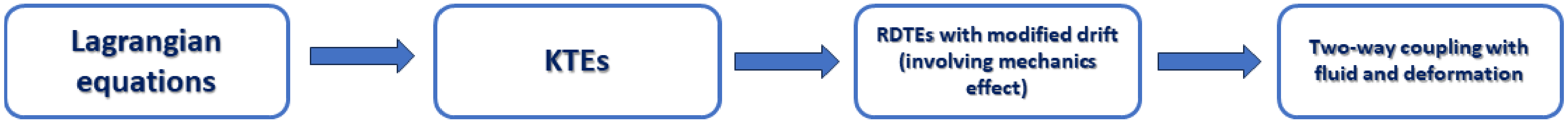
$$c_1(x,0) = c_{10}(x) > 0, \quad c_2(x,0) = c_{20}(x) > 0$$
$$h(x,0) = h_0(x) > 0, k(x,0) = k_0(x) > 0, \quad x \in \Omega,$$

Theoretical results

- Global existence of weak solutions for $n = 3$.
- Turing instability with respect to haptotactic sensitivity b_1.

Outlook

- Bio-reactor experiments to include mechanical effects on (de) differentiation.
- More careful modeling of mechanical and tactic effects on microscale

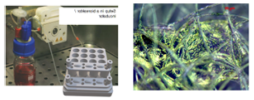


- Including detailed information about scaffold, possible effect of porosity and stiffness.

Work flow

Experimental Study

Scaffold production and cell-seeding → **Analysis** →



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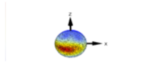
Theoretical Study

Image analysis



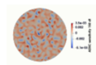
Claudia Redenbach, Alex Keilmann

Modeling and analysis

- PDE-ODE system for cells, hyaluron and ECM,
- fluid(Stokes),
- deformation of tissue

Christina Surulescu, Nishith Mohan

Numerical simulations



Bernd Simeon, Elise Grosjean

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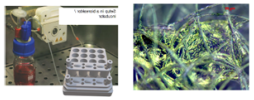
Work flow

Experimental Study

Scaffold production and cell-seeding → **Analysis** →



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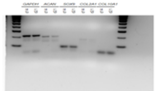

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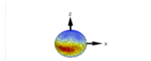

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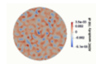

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Forward simulations
Sensitivity analysis
Parameter identification
Simulations costs reduction

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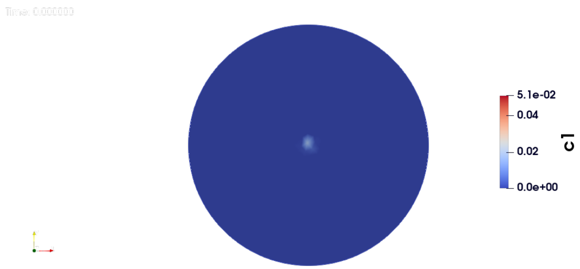
Simulations of tissue regeneration

3D simulations (FreeFem++ with PETSc)

Parallel simulations of a problem of tissue regeneration

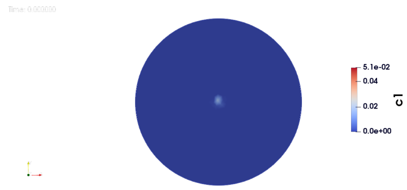


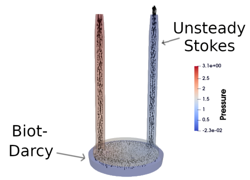
Adipose stem cells density

Industrial tissue

Simulations of tissue regeneration

3D simulations (FreeFem++ with PETSc)
How the parameters influence the models?





Industrial tissue

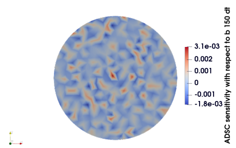
E. Grosjean, B. Simeon, C. Surulescu. A mathematical model for meniscus cartilage regeneration (preprint, 2023)

Sensitivity analysis

Sensitivity analysis calculates the **rates of change** in the output variables of a system which result from **small perturbations** in the problem parameters.

$$\mathscr{P} : \boldsymbol{\mu} \to u(\boldsymbol{\mu}),\ \boldsymbol{\mu} = (\mu_1, \ldots, \mu_n)$$

Sensitivities: $\frac{\partial u}{\partial \mu_j}(\mathbf{x}; \boldsymbol{\mu}^s),\ j = 1, \ldots, n.$



ADSC' density sensitivity with resp. to b_1 (150 dt)

Diffusion

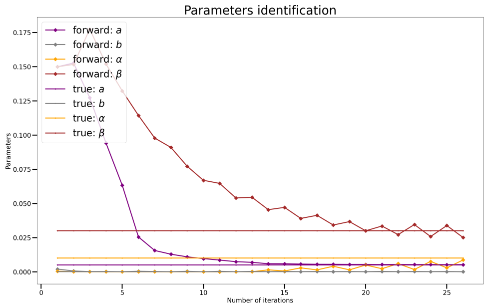
Haptotactic parameter

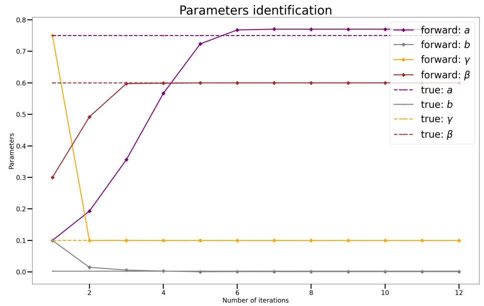
Differentiation parameters

How to find $\boldsymbol{\mu}^s$?

Parameters identification

Identification of a, b, α, β with Gauss-Newton algorithm (Tikhonov regularization) on $\Omega = [0,1] \times [0,1]$, $T = 1$



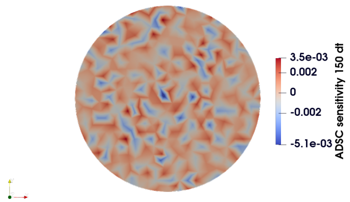


- ◇ Identification successful provided initial guess not too far
- ◇ Need experimental values

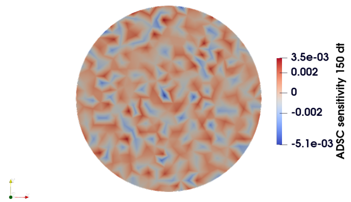
NIRB on the sensitivity equations

How do we reduce the time simulations of the sensitivity equations?

Grosjean E., and Simeon, B. (2023). The non-intrusive reduced basis two-grid method applied to sensitivity analysis, Preprint.



(a) FEM fine sensitivities after 150 time steps



(b) NIRB approximation with $N = 40$

- Accurate results with online time saving
- Many training solutions required

Conclusion & perspectives

Conclusion

- Forward simulations[1 2]
- Loosely coupling between the two models[2]
- Sensitivity analysis of two models: Cells density and bioreactor models (2nd talk)
- New methodology for the sensitivity to reduce simulations costs[3]

Perspectives

- Models simplification
- Validation with measures (1rst talk)
- Enhancement of our NIRB method[4 5]
- Other sensitivity evaluations (e.g. Sobol indices)

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[1] Simeon, B., Die Macht der Computermodelle: Quellen der Erkenntnis oder digitale Orakel? (2023)
[2] Grosjean E., Simeon, B. The non-intrusive reduced basis two-grid method applied to sensitivity analysis, preprint, 2023
[3] Grosjean, E. Simeon, B., Surulescu. C. A mathematical model for meniscus cartilage regeneration, preprint, 2023
[4] Maday, Y., Stamm, B. Locally adaptive greedy approximations for anisotropic parameter reduced basis spaces (2013)
[5] Barnett, J. L., Farhat, C., Maday, Y. Mitigating the Kolmogorov Barrier for the Reduction of Aerodynamic Models using Neural-Network-Augmented Reduced-Order Models (2023)

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Thank you for your attention!


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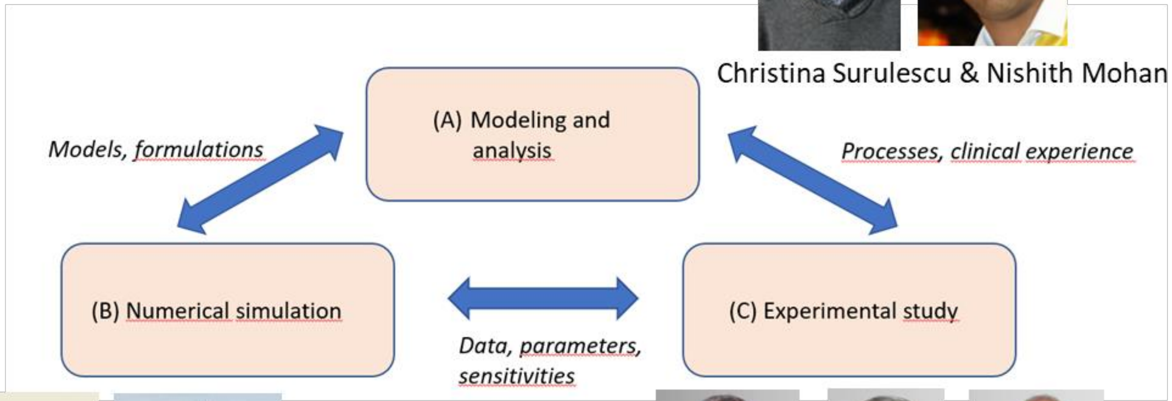


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